VELSICOL
CHEMICAL CORPORATION

Christopher Charniak
Regulatory Compliance Specialist
Email: ccharniak@velsicol.com
Phone: (847) 635-3448
Fax: (847) 298-9844

201-16656

November 20, 2007

Mr. Stephen L. Johnson,
Administrator
U.S. Environmental Protection Agency
Ben Franklin Post Office
P.O. Box 862
Washington, DC 20044
Attention: Chemical Right-to-Know Program

Mr. Johnson:

Please find below a response to your July 25, 2002 letter describing EPA's comments to Velsicol Chemical Corporation's HPV Challenge Robust Summary submission for isodecyl benzoate

1.) Chemistry (melting point, boiling point, vapor pressure, water solubility, and partition coefficient)

The water solubility test was conducted in a pH range of 4.38 to 5.98 instead of the required environmental pH of 7.0. The submitter needs to conduct a water solubility test using a hardness of <180 mg/L $CaCO_3$ and a pH of 7.0 to mimic environmental test condition and help explain the ecological test results.

Velsicol Chemical Corporation's water solubility test for the isodecyl benzoate report was done in accordance with the European Directive 96/69/EEC-A.6. This Directive's methods are based on the OECD Test Guideline 105, the recommend test method for the HPV Program. Approximately 0.050 grams of isodecyl benzoate were added to 400 milliliters of double glass-distilled water in a flask. The pH values of the samples were calculated after the flasks and been heated, stirred, cooled till equilibrium, and finally filtered.

The low pH values for the samples appear to be caused by the filtration step, since the pH values for blank samples were similar. Although the EPA suggests that another water solubility test be run at mock environmental conditions (pH 7.0), Velsicol Chemical Corporation believes that is not necessary since the effect of pH on the water solubility of a unionisable molecule will be the same at each pH value tested.

2.) ***Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)***

<u>Biodegradation</u> –Results from two tests are substantially different. The submitter needs to explain the conflicting results. The submitter also needs to characterize the material tested as to the distribution of molecular weights and alkyl chain branching. Differences in composition between the test substances might account for the divergent results.

The material used in the two biodegradation tests was highly pure (> 98%) isodecyl benzoate. The molecular weight and alkyl chain branching would have been similar for both test materials. Therefore, Velsicol Chemical Corporation believes the divergent results of the two biodegradation tests are the result of critical differences in the two test methods used.

The biodegradation test that resulted in isodecyl benzoate being characterized as not readily biodegradable was conducted under the OECD Guideline for Testing of Chemicals No. 301D. These tests were conducted at 20±1°C in a closed system with a sample of activated sludge that was filtered and aerated for 24 hours.

The other biodegradation test was conducted under the methods of Modified MITI Test (I) [OECD 301C]. These tests were run at 25±2°C in a closed system in which the activated sludge was aerated then one third was removed and an equal volume of dechlorization water was added to the remaining portion and aerated again. Then a synthetic sewage, consisting of glucose, peptone, and monopotassium phosphate at 5 (W/V)% that was dissolved in dechlorization water respectively and pH adjusted to 7.0 using sodium hydroxide, was added at 0.1 (W/V)%.

There are significant differences in the methods used for the two biodegradation tests that probably lead to the discrepancy in the final determination. The divergent test results are most likely due to a difference in inoculum levels between the OECD 301C and 301D tests.

3.) ***Health Effects (acute toxicity, repeat dose toxicity, genetic toxicity and reproductive/developmental toxicity)***

Reproductive Toxicity: The submitter concluded that the reproductive toxicity endpoint is addressed by evaluation of reproductive organs in the 28-day repeated-dose toxicity study and the availability of a developmental toxicity study. EPA disagrees with this conclusion because EPA's guidance specifically states that when effects on reproductive organs have been sufficiently documented in an existing 90-day repeated-dose study and a developmental toxicity study is available, the reproductive toxicity endpoint can be considered addressed for the purposes of the HPV Challenge Program. The 28-day study is not of a sufficiently long duration for assessing effects on the reproductive organs. Therefore, a reproductive toxicity screen study (OECD TG 421) is necessary to address this endpoint.

As requested by the EPA, Velsicol Chemical Corporation will conduct a reproductive toxicity screen study (OECD TG 421) next year in order to address the reproductive toxicity endpoint. Velsicol will submit a summary of these results when they become available.

4.) ***Ecotoxicity (fish, invertebrates, algae)***

The submitted invertebrate chronic robust summary failed to provide an LOEC at the chemical's aqueous water solubility limit. Adding to this problem is that the submitted water solubility test was not done at pH 7.0. Because the chemical's measured log K_{ow} value is 4.2, and analogous chemicals have shown chronic effects, EPA believes that the chemical may exhibit chronic aquatic toxicity at the true aqueous water solubility limit. Thus, the submitter needs to provide relevant water solubility data to conclude if indeed the chemical was tested at the aqueous water solubility limit in the daphnia chronic study. For this reason, EPA recommends conducting a water solubility test under environmental conditions including a pH of 7 and a hardness of < 180 mg/L as $CaCO_3$. EPA recommends redoing the chronic invertebrate study if the chemical was not tested at the aqueous water solubility limit. Because this chemical is difficult to test due to its low water solubility, guidance on how to test chemicals according to the OECD Guidance Document on Aquatic toxicity testing of Difficult Substances and Mixtures is available at http://www.oecd.org/ehs/test/monos.htm. The submitter supplied a fish chronic study. This fish study has similar problems as the submitted daphnia chronic study.

Velsicol Chemical Corporation has addressed the EPA's concerns regarding the water solubility test conducted above. The water solubility of isodecyl benzoate was found to be less than 68.6 µg/L. Tests were run at isodecyl benzoate concentrations of 0.81, 2.7, 9.0, 30, and 100 µg/L in the chronic toxicity studies for both fish and daphnia. Therefore, Velsicol Chemical Corporation believes the chronic fish and daphnia studies were in fact run below, near, and above isodecyl benozate's true aqueous water solubility limit.

SPECIFIC COMMENTS ON THE ROBUST SUMMARIES

Health Effects:

Statistical methods should be identified in the robust summaries for acute inhalation, repeated dose, developmental, and genetic toxicity.

Acute Inhalation Toxicity: Behrens-Reed-Muench method.

Reference: Thakur, AK, Fezio, WL: A computer program for estimated LD_{50} and its confidence limits using a modified Behrens-Reed-Muench cumulate method. Drug and Chemical Toxicology, 4: 297-305, 1981

Developmental Toxicity:

Statistical Test	Parameter
Chi-square test with Yates' correction factor	Fetal Sex Ratios
Fisher's Exact test	Malformations and Variations
Mann-Whitney U-test	Early and late Resorptions, Dead Fetuses, Post Implantation Losses, Mean Litter Proportions of Malformations and Variations
One-way ANOVA with Dunnett's test	Corpora Lutea, Total Implantations, Viable Fetuses, Fetal body Weights, Maternal Body Weights and Weight Changes, Maternal Net Body Weight Changes, Gravid Uterine Weights, Maternal Food Consumptions

Reference: BMDP (1979) biomedical Computer Programs. (Dixon, WJ and Brown, MB, eds.) University of California Press, Berkeley, CA, pp. 612, 780, 781.

Repeated Dose Toxicity:

All statistical analyses were carried out separately for males and females using the individual animal as the basic experimental unit.

The following sequence of statistical tests was used for bodyweight gains, organ weight and clinical pathology data:

- If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%) the proportion of values different from the mode was analyzed by Fisher's exact test[1] followed by Mantel's test for a trend in proportions[2]. Otherwise:
- Bartlett's test[3] was applied to test for heterogeneity of variance between treatments. If significant heterogeneity was found at the 1% level, a logarithmic transformation was tried to see if more stable variance structure could be obtained.
- If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out followed by Williams' test[4] for a dose related response.

---

[1] Fisher, RA (1950) *Statistical Methods for Research Workers,* 11th ed., Oliver and Boyd, Edinburgh.
[2] Mantel, N (1963) *J. Amer. Statist. Ass.,* **58**, 690.
[3] Bartlett, MS (1937) *Proc. Roy. Soc.,* **A 160**, 268.
[4] Williams, DA (1971/2) *Biometrics,* **27**, 103 and **28**, 519.

- If significant heterogeneity of variance was present and could not be removed by a logarithmic transformation, the Kruskal-Wallis analysis of ranks[5] was used. This analysis was followed by the non-parametric equivalent of the William's test (Shirley's test[6]).

Covariate analysis of organ weight data (with final bodyweight as covariate) was also performed using adjusted weights for organs where a correlation between organ weight and bodyweight was established at the 10% level of significance.[7]

Significant differences between control animals and those treated with the test substance have been expressed at the 5% (*P < 0.05) or 1%(**P < 0.01) level.

Genetic Toxicity:

- For the Genetic Bacterial *In Vitro* Test:

  The mean number of revertant colonies for all treatment groups is compared with those obtained for solvent control groups. The mutagenic activity of a test substance is assessed by applying the following criteria:

  A) If treatment with a test substance produces an increase in revertant colony numbers of a least twice the concurrent solvent controls, with some evidence of positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S-9 mix, it is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

  B) If treatment with a test substance does not produce reproducible increases of at least 1.5 times the concurrent solvent controls, at any dose level with any bacterial stain, it is considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.

  c) If the results obtained fail to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs A and B, the following approach is taken in order to resolve the issue of the substance's mutagenic activity in this test system:

     i) Repeat tests may be performed using modification of the experimental method. These modification include (but are not restricted to), the use of a narrower dose range than that already tested' the use of different levels of liver homogenate S-9 fraction in the S-9 fraction in the S-9 mix. Should an increase in revertant colony numbers be observed which satisfies paragraph A the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

---

[5] Kruskal, WH and Wallis, WA (1952/3) *J. Amer. Statist. Ass.*, **47**, 583 and **48**, 907.
[6] Shirley, E (1977) *Biometrics*, **33**, 386.
[7] Angervall, L and Carlstrom, E (1963) *J. Theoret. Biol.*, **4**, 254.

ii) If no clear "positive" response can be obtained the test data may be subjected to analysis to determine the statistical significance of any observed increases in revertant colony numbers. The statistical procedures used will be those described by Mahon *et al.*[8] and will usually be analysis of variance followed by Dunnett's test.

- For the Genetic Non-Bacterial *In Vitro* Test:

  The number of aberrant metaphase figures in each treatment group was compared with the solvent control value using Fisher's test.[9]

  A positive response is claimed if an increase in the number of aberrant cells is observed at least at one dose level, and is so substantially greater than the laboratory negative control range that formal statistical analysis is deemed unnecessary. Evidence of a dose relationship is usually required.
  A negative response is claimed if no statistically significant increase in the number of aberrant cells above concurrent control frequencies is observed, at any dose level.

  A further evaluation may be carried out if a statistically significant increase in the number of aberrant cells above concurrent control frequencies is observed, at least at one dose level, which is not substantially greater than the laboratory negative control range.

- For the Genetic *In Vivo* Test:

  Non-parametric statistical methods, based on rank are chosen for analysis of results because:

  a) They are suited to analysis of data consisting of discrete/integer values such as the incidence of micronucleated polychromatic erythrocytes.
  b) The methods make few assumptions about the underlying distribution of data and therefore the values do not require transformation to fit a theoretical distribution (where data can be approximately fitted to a normal distribution, the results of non-parametric analysis and classical analysis of variance are very similar).
  c) "Outliers" are frequently found in the polychromatic erythrocyte to normochromatic erythrocyte ratios for both control and treated animals; non-parametric analysis does not give such values an undue weighting

---

[8] Mahone, GAT, Green MHL, Middleton, B Mitchell, IDEG, Robinson, WD and Tweats, DJ (1989) Analysis of data from microbial colony assays in Kirkland, DJ ed. *UKEMS Subcommittee on Guidelines for Mutagenicity Testing. Report, Part III. Statistical Evaluation of Mutagenicity Test Data,* p.26. Cambridge University Press, Cambridge.

[9] Fisher, RA (1973) The Exact Treatment of 2 x 2 Table in: *Statistical Methods for Research Workers.* Hafner Publishing Company, New York.

Unless there is a substantial difference in response between sexes (which is rare) results for the two sexes are combined to facilitate interpretation and maximize the power of statistical analysis. For a comparison of an individual treated group with a concurrent control group, Wilcoxon's sum of ranks test is used.[10 & 11]

Acute Toxicity: The robust summary for acute inhalation toxicity did not include the frequency of clinical observations, necropsy findings (if performed), or relationships between sub-lethal clinical signs and exposure concentration.

- *The robust summary for acute inhalation toxicity did not include the frequency of clinical observations*

  According to the text of the acute inhalation toxicity study, observations during exposure were conducted for gross signs of toxicological or pharmacological effects hourly. Detailed physical examinations were conducted prior to and at approximately 30 to 60 minutes after exposure and then on a daily basis until the mice were found dead or terminally sacrificed. The checks for mortality were done twice a day.

- *The robust summary for acute inhalation toxicity did not include necropsy findings (if performed)*

  According to the text of the acute inhalation toxicity study, "Gross postmortem examinations were performed on all animals. The external surface, as well as the thoracic, abdominal, and cranial cavities and their organs and tissues were subject to gross examination." These studies revealed "respiratory lesions and alopecia which were considered to probably be treatment-related."

- *The robust summary for acute inhalation toxicity did not include relationships between sub-lethal clinical signs and exposure concentration.*

  The robust summary described "adverse clinical signs at all test levels included languid behavior, rough haircoat, dyspnea, polypnea, squinting tremors, and hunched appearance." The frequency and duration of these sub-lethal clinical signs were less at the lower concentration test levels.

---

[10]Hollander, M and Wolfe, DA (1973) *Non-parametric Statistical methods.* J. Wiley and Sons, New York & London.

[11] Langley, R (1979) *Practical Statistics,* 2nd edition. Pan Books, London and Sydney.

Repeated-dose Toxicity: The robust summary did not indicate the number of rats per dose level that displayed signs of toxicity or whether observed behavioral effects were reversible.

- *The robust summary did not indicate the number of rats per dose level that displayed signs of toxicity*

  There were no treatment-related effects seen at the low dosage of 15 mg/kg/day. The only treatment-related finding at the intermediate dosage level of 150 mg/kg/day was the microscopic finding of eosinophilic inclusions in kidney tubules found in four males. The treatment-related effects seen at the high dosage level of 1000 mg/kg/day were:
  - Centrilobular hepatocyte enlargement was found in 4 male and 3 female rats.
  - Eosinophilic intracytoplasmic droplets in proximal convoluted tubules were found in the 5 male rats.
  - The macroscopic post mortem examination performed at termination revealed enlarged livers for 4 male and 2 female rats.
  - Clinical signs, such as altered appearance, palpbral closure in the area, tremors, increased hindlimb grip strength, or increased hindlimb splay, were observed for all rats, but the females appeared to be more affected than the males.

- *The robust summary did not indicate whether observed behavioral effects were reversible.*

  The "Isodecyl Benzoate: Twenty-Eight Day Oral Toxicity Study in the Rat with Functional Observational Battery" report does not make any conclusions as to whether or not the observed behavioral effects are reversible.

Developmental Toxicity: The robust summary did not indicate the frequency of clinical observations or incidences of developmental effects.

- *The robust summary did not indicate the frequency of clinical observations*

  The toxicity report states that: "All rats were observed twice daily for morbidity and mortality. Detailed clinical observations were recorded individually from days 0 through 20 of gestation. Observations were recorded before dosing during the dosing period. [*Sic*] Animals were also observed for signs of toxicity approximately one hour following treatment throughout the dosing period. All significant findings were recorded at the post-dosing observation periods."

- *The robust summary did not indicate the incidences of developmental effects.*

> "Developmental toxicity was exhibited at a dose level of 1000 mg/kg/day by a decrease in mean foetal bodyweight and a reduction in incidence of cervical centrum no. 1 ossified."

Ecotoxicity Studies:

Fish: Missing acute study details include pH, water hardness, temperature, DO, solvent used and if the chemical test concentration was measured.

> The temperature for this experiment was set at 12±1°C. The pH range for the experiment was 8.0 to 8.3 with a mean of 8.1. The water used in the testing was taken from a well and characterized as "medium-hard." The water had hardness values ranging from 132 to 144 mg/L $CaCO_3$ with a mean of 137 mg/L $CaCO_3$. The report notes that: "Water pH was consistent with values for medium-hard water." The dissolved oxygen content ranged from 8.8 to 9.2 mg/L during the testing, which corresponded to dissolved oxygen concentrations exceeding 60% of saturation throughout the testing. The solvent dimethyl formamide was introduced to the mixing chamber to aid in the solubilization of isodecyl benzoate. The samples of test substances were measured to have concentrations ranging from 4.6 to 8.4 mg of isodecyl benzoate per liter. The mean concentration was found to be 6.5 mg/L.

Invertebrates: Missing details include pH, hardness, temperature, DO, and type of solvent and concentration.

> The pH ranged from 8.1 to 8.5 during the testing period. The temperature range for the testing period was 19.7 to 20.5°C. The dissolved oxygen content ranged from 8.0 to 8.4 mg/L during the testing period. The water used in the testing came from a well and was characterized as medium-hard. The water hardness value was found to be 136 mg/L $CaCO_3$. The type of solvent used was dimethyl formamide. The mean measured concentrations used in the testing were: 0.089, 0.11, 0.28, 0.46, and 0.70 milligrams isodecyl benzoate per liter.

Algae: Missing details include pH, hardness, temperature, type of solvent if used and temperature. [Sic]

> The solvent used in these test was dimethyl formamide. The temperature for the test chamber was set at 24±2°C. The pH measurements ranged from 7.4 to 9.1. The hardness of the water was not stated in the report. The water came from the same well source as the fish and invertebrates studies (which listed the water as medium-hard; 132 to 144mg/L $CaCO_3$), however, the water was purified using reverse osmosis for this experiment.

Invertebrate Chronic: Missing study details include an indication if a solvent was used and concentration, DO, pH and water hardness. There appears to be a discrepancy in the method and remarks section for lowest concentration tested and what is stated in the results section.

- *Missing study details include an indication if a solvent was used and concentration, DO, pH and water hardness*

  The type of solvent used to aid the solubilization of isodecyl benzoate was dimethyl formamide at stock concentrations of 0.30, 0.090, 0.027, and 0.0081 mg isodecyl benzoate per milliliter. During the testing period the water temperatures ranged from 19.0 to 20.4°C. The pH of the water ranged from 8.2 to 8.6 and the dissolved oxygen content ranged from 8.0 to 9.0 mg/L. The well water that was used was classified as medium-hard and had a hardness measurement ranging from 136 to 144 mg/L $CaCO_3$.

- *There appears to be a discrepancy in the method and remarks section for lowest concentration tested and what is stated in the results section.*

  The toxicity report states: "Nominal concentrations selected for use in this study were 0.81, 2.7 9.0 30 and 100 µg isodecyl benzoate per liter. On Day 0, samples collected from the control and the three lowest treatment groups were below the limit of quantitation (LOQ), 6.0 µg/L, while samples collected from the two highest treatment groups were 49 and 58% of nominal respectively. Since the two lowest treatment groups were below the LOQ, no further analyses were performed for these groups. However, since the nominal concentration in the third highest group (9.0 µg isodecyl benzoate per liter) was greater than the LOQ, sampling continued throughout the test. The mean measured concentrations of the two highest these concentrations were 10 and 39 µg isodecyl benzoate per liter. In the lower three test levels (0.81, 2.7, and 9.0 µg isodecyl benzoate per liter) all measurements were less than the LOQ. Mean measured concentrations were used in the estimation of the NOEC, LOEC, MATC and 21-day EC50 values."

  The apparent discrepancy in the concentration of the two test substances with the most isodecyl benzoate is due to a difference in the nominal and measured concentration. The nominal concentrations were listed in the report as 30 and 100 µg isodecyl benzoate per liter, while the measured concentrations of these test substances had mean values of 10 and 39 µg isodecyl benzoate per liter.

There are no other modifications at this time to Velsicol Chemical Corporation's HPV submission for isodecyl benzoate. In addition to the reproductive OECD TG 421 test, Velsicol Chemical Corporation intends to provide the EPA with endpoints for photodegradation, water solubility, and transport once the test data for these environmental fate topics become available as was indicated in HPV testing plan. Please feel free to contact me at (847) 635 3448 with any additional comments.

Regards,

Christopher Charniak
Regulatory Compliance Specialist